

Full Length Research Paper

# Inhibition of angiotensin converting enzyme by *Rhazya stricta*, *Moringa peregrina* and *Achillea fragrantissima*, used in traditional system of medicine in Arabian Peninsula: Implication in the management of hypertension

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In recent years, there has been a marked increase in the popularity of medicinal plants derived from the traditional sources of knowledge. This is in congruence to the associated benefits of ease of accessibility and low cost of disease management. Additionally, most of the potent drugs being used currently for chronic diseases are derivatives of natural products. In the current study, the potential of hydro-alcoholic extracts of *Rhazya stricta*, *Moringa peregrina* and *Achillea fragrantissima* to inhibit angiotensin converting enzyme (ACE) which is involved in the etiology of hypertension and cardiovascular disorders, were examined. The herbal derivatives of these plants have been used for a long time now, in traditional systems of various ethno-pharmacological cultures in Arabian Peninsula and worldwide. The study also evaluated the anti-oxidative properties of these extracts which are reflected by their ability to inhibit DPPH radicals. The phytochemical analysis which focused on the tannin contents of the three extracts demonstrated a direct proportionality between the tannin content and the ACE inhibition activity of the tested extracts in the order: *R. stricta* > *M. peregrina* > *A. fragrantissima*. The results partially support the use of these plants as nutraceuticals and warrant the need for further studies on isolation and characterization of the bioactive constituents of these plants that are responsible for the associated pharmacological property of inhibiting ACE.

**Key words:** Medicinal plants, hypertension, angiotensin converting enzyme, oxidative stress.

## INTRODUCTION

Elevated blood pressure is currently one of the leading risk factors for disease and disability worldwide (Rahimi

et al., 2015). Angiotensin converting enzyme (ACE) is a key component in the renin angiotensin aldosterone

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system which is involved in the regulation of blood pressure by cleaving angiotensin I to angiotensin II which is a potent vasoconstrictor (Balasuriya and Rupasinghe, 2011). The enzyme also restricts the vasodilatory and natriuretic properties of bradykinin by catalyzing its degradation to inactive components, thereby further contributing in the physiological manifestation of vascular dynamics of blood flow. Since, the activating cascade of this system is associated with vascular hypertension, ACE inhibition has become a major target for management of hypertension (Hansen et al., 1995). ACE inhibitors prevent the formation of angiotensin II by ACE and thereby reduce peripheral vascular resistance and blood pressure. Although, ACE inhibitors currently available such as captopril are generally effective in reducing blood pressure, their efficacy appear to vary between different ethnic groups and age levels (Brown and Vaughan, 1998).

Moreover, it is being increasingly considered safer to apply nutritional approach that relies on dietary habits and traditional herbal medicines to treat chronic diseases like diabetes and hypertension (Liu et al., 2003; Ullah et al., 2015). It has been reported that more than 50% of drugs used in modern medicine are isolated from herbs or derived from modification of phytochemicals (De Smet, 2002). Recent studies have stated that 75-90% (developing world) and 80% (less developed/developing countries) of the current world population relies on the use of herbal medicines for their primary health care (Robinson and Zhang, 2011; Mehta et al., 2015). Bioactive components of these herbal derivatives such as terpenoids and polyphenolic compounds including flavonoids, hydrolysable tannins, xanthenes, procyanidins, caffeoylquinic acid derivatives have been found to be effective as natural ACE inhibitors (Kang et al., 2003; Loizzo et al., 2007).

In the light of the developing interests in the pharmacology of natural products such as ACE inhibitors, the present study has examined three plants with ethno-medicinal significance in Arabian Peninsula for the presence of ACE inhibitory and anti-oxidative properties. The study also demonstrates the presence of tannins as an important molecule which might be responsible for the observed pharmacological properties. Plants such as *Rhazya stricta*, *Moringa peregrina* and *Achillea fragrantissima* are used in folk medicine for the treatment of various ailments, including chronic and pathogenic diseases like diabetes (Ullah et al., 2015), inflammatory conditions (Ali et al., 1998) and microbial infections (Hammad et al., 2014).

## MATERIALS AND METHODS

### Materials

Fresh leaves of *R. stricta*, *A. fragrantissima* and *M. Peregrina* were procured from the local market supplying plants popular for traditional and medicinal value (Tabuk, KSA). These were

authenticated by a plant taxonomist from the Department of Biology, Faculty of Science, University of Tabuk. Methanol and ultrapure water were purchased from Thermo Fisher Scientific (Fremont, CA, USA). Tannic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was delivered by Fluka (USA). Angiotensin converting enzyme from porcine kidney, its substrate N-Hippuryl-L-histidyl-L-leucine hydrate, standard hippuric acid and PBS were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Captopril was purchased from Calbiochem, Germany. All other chemicals and reagents were of analytical grade.

### Extraction procedure

The extraction procedure was followed as described by Suarez et al. (2010), with slight modifications. The leaves were rinsed with cold water and dried under shade. Dried plant material was crushed into small pieces in wooden mortar and pestle and further ground to fine dry powder with a blender. A quantity of 300 g dry leaves powder was then soaked in 2 L of 80% methanol in a conical flask placed in a water bath at 40°C for 24 h with constant shaking. The extracted mixture was then filtered through a double layered clean cheese cloth and subsequently through double layered Whatman paper. The filtrate was concentrated under reduced pressure at 35°C using a Buchi rotavapor R-210 (Switzerland). Finally, the concentrated extract was further placed under vacuum at -30°C for 3-4 days to yield solid/thick pastes. The residual material of *R. stricta*, *A. fragrantissima* and *M. Peregrina* was weighed 54, 38 and 45 g, providing total yields of 18, 12.6 and 15% (w/w), respectively.

### Angiotensin converting enzyme inhibition assay

ACE inhibition assay was carried out as per Hooper et al. (1987) with modifications. In brief, a pre-incubation mixture contained 100 mM Tris-HCl buffer with 300 mM NaCl and 10  $\mu$ M ZnCl<sub>2</sub>, pH 8.3/positive control/test sample of various concentrations and 2 mU of ACE enzyme. The reaction mixture was mixed and pre-incubated at 37°C for 10 min. Following pre-incubation, substrate (N-Hippuryl-L-histidine-L-leucine tetrahydrate) was added to a final concentration of 5 mM. The reaction mixture was mixed and incubated at 37°C for 30 min. The reaction was arrested by boiling in water bath for 4 min. A control reaction was also carried out without the test samples. The reaction mixture was centrifuged at 15,000 rpm for 10 min at 25°C. The supernatant was transferred to HPLC vials and subjected to HPLC analysis. The product (hippuric acid) and other components were separated by reverse phase HPLC method using a C18 column. HPLC was performed using Shimadzu Model LC-2010AHT (Shimadzu Corporation, Tokyo, Japan). Briefly, the stationary phase used was an octadecylsilane column (Phenomenex Luna® HPLC column, C18, 5  $\mu$ m, 250 x 4.6 mm). The mobile phase consisted of gradient mixture of HPLC buffer (A) and acetonitrile (B), which were separately filtered through filter (0.45 $\mu$ m, Pall India Pvt Ltd., India) and degassed by sonication for 3 min. The HPLC column was allowed to equilibrate for 60 min before the start of analysis. Separation was carried out with 10 min isocratic elution (70% A and 30% B). The flow rate of mobile phase was maintained at 1.5 ml/min throughout the analysis and detector wavelength was kept at 230 nm for detection of hippuric acid.

### Anti-oxidant activity by DPPH radical scavenging assay

Antioxidants reduce DPPH free radical to 2,2-diphenyl-1-picryl hydrazine, a colorless compound. DPPH assay was carried out as per the method of Vani et al. (1997). In brief, the total reaction mixture contained methanol/positive control/various increasing

**Table 1.** IC<sub>50</sub> values of the tested hydro-methanolic extracts for ACE inhibition and DPPH radical scavenging activity.

Extract	ACE Inhibition {IC <sub>50</sub> (µg/ml)}	DPPH radical Inhibition {IC <sub>50</sub> (µg/ml)}
<i>R. stricta</i>	3015.83±1.24	417.24±1.63
<i>M. peregrina</i>	3705.78±0.98	67.99±0.85
<i>A. fragrantissima</i>	5254.95±2.78	95.91±0.52

Analysis was done in three parallel determinations. The IC<sub>50</sub> values were determined by logarithmic regression and are presented together with their respective 95% confidence limits.

concentrations of standard compound/test extract (as shown in the figures) and DPPH to a final concentration of 0.132 mM. The reaction mixture was incubated at 25°C for 20 min and absorbance was read at 510 nm using UV-spectrophotometer. The control reaction was carried out without test extracts which were replaced by appropriate volumes of the vehicle. Ascorbic acid was used as a standard compound (results not shown). Results were presented as % inhibition of radicals (compared to control without any test agent).

#### Analysis of tannin content

The extract was subjected to analysis for the presence of tannins. Quantitative determination of tannin was done using tannic acid as a reference compound (Makkar, 2000).

#### Statistical analysis

All experiments were performed in three different sets, with each set in triplicate. The data are expressed as mean ± SEM (standard error of the mean). Statistical analysis was performed for analysis of variance (ANOVA) followed by F-test using SPSS version 11.5 (SPSS, Inc., Chicago, IL). Values of P, which were ≤0.05, were considered significant.

## RESULTS AND DISCUSSION

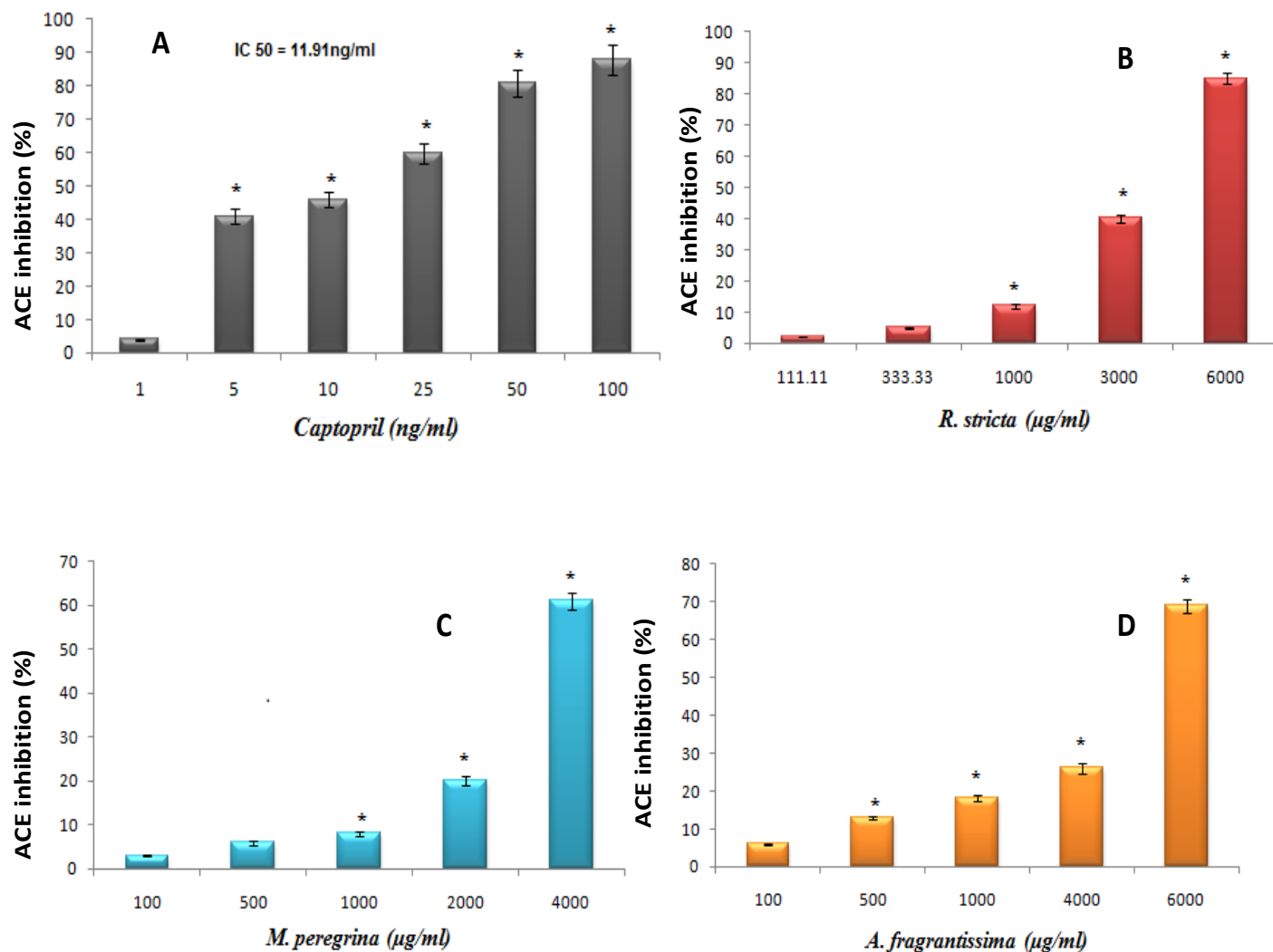
### Tested extracts of *R. stricta*, *M. Peregrina* and *A. fragrantissima* showing progressive inhibition of mammalian angiotensin converting enzyme

As presented in Figure 1B, C and D, the hydro-methanolic extract of the three tested extracts of *R. stricta*, *M. Peregrina* and *A. fragrantissima* showed a dose-response with progressive inhibition of ACE activity proportionate to the increase in the extract concentration. The standard anti-hypertensive drug captopril was used as a reference for the standardization of the assay as shown in Figure 1A. The anti-ACE activity of the three extracts were demonstrated by different inhibition potential as shown in Table 1, which provides the IC<sub>50</sub> values reflecting the efficiency of the extracts in the following order: *R. stricta* > *M. peregrina* > *A. fragrantissima*. Hypertension is the most common cardiovascular disease and a major health issue in both developed and developing countries. There have been a

number of treatment modalities for hypertension that include the use of diuretics, β-blockers, calcium channel blockers and angiotensin II receptor blockers. However, the most common of these are angiotensin converting enzyme inhibitors. ACE inhibitors have wide therapeutic potential in the treatment of heart failure and high blood pressure. Randomized, placebo-controlled trials have shown that ACE inhibitors are effective in lowering blood pressure and in the treatment of cardiovascular dysfunction (Pitt et al., 2000). Recent studies have shown the emergence of a number of plant-derived extracts with potential to provide lead molecules for the synthesis and development of new anti-hypertensive agents (Chaudhary et al., 2015; Gasparotto et al., 2011).

### DPPH radical inhibition profile of the extracts showing progressive dose-response curve

Substantive data indicate that reactive oxygen species (ROS) and oxidative stress are important elements of cardiovascular diseases including atherosclerosis, hypertension and congestive heart failure (Sugamura and Keaney, 2011). Low density lipoprotein complexes (LDL) contain lipid species that are subjected to oxidation in the presence of several ROS known to exist in vascular wall (Sorescu et al., 2001). Oxidative modification of LDL is known to be a feature of the atherosclerotic process (Witztum and Steinberg, 2001). In hypertensive patients, angiotensin II increases chronically and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated, which causes a rise in ROS (Sharifi et al., 2013). In consideration of these observations, a multi-dimensional approach requires a herbal candidate which has pleiotropic action mechanisms that could target various factors involved in the etiology of cardiovascular disorders. In the experiment presented in Figure 2A, B and C, the antioxidant capacity of the three extracts was evaluated using DPPH radical scavenging assay. As shown in the figure, these extracts led to the progressive scavenging of DPPH radical in a dose-dependent manner. However, it is the *M. peregrina* extract which seems to be idealistic in addition to its ACE inhibition activity, it also possess the most potent anti-oxidative properties as compared to the other two extracts (Table 1).



**Figure 1.** Inhibition profile of mammalian angiotensin inhibiting enzyme by (A) Standard drug captopril and the hydro-methanolic extracts of (B) *R. stricta*, (C) *M. peregrina* and (D) *A. fragrantissima*. Values reported are  $\pm$  SEM of three independent experiments. \* $P \leq 0.05$ : significant when compared with the control (in the absence of tested concentrations of extract).

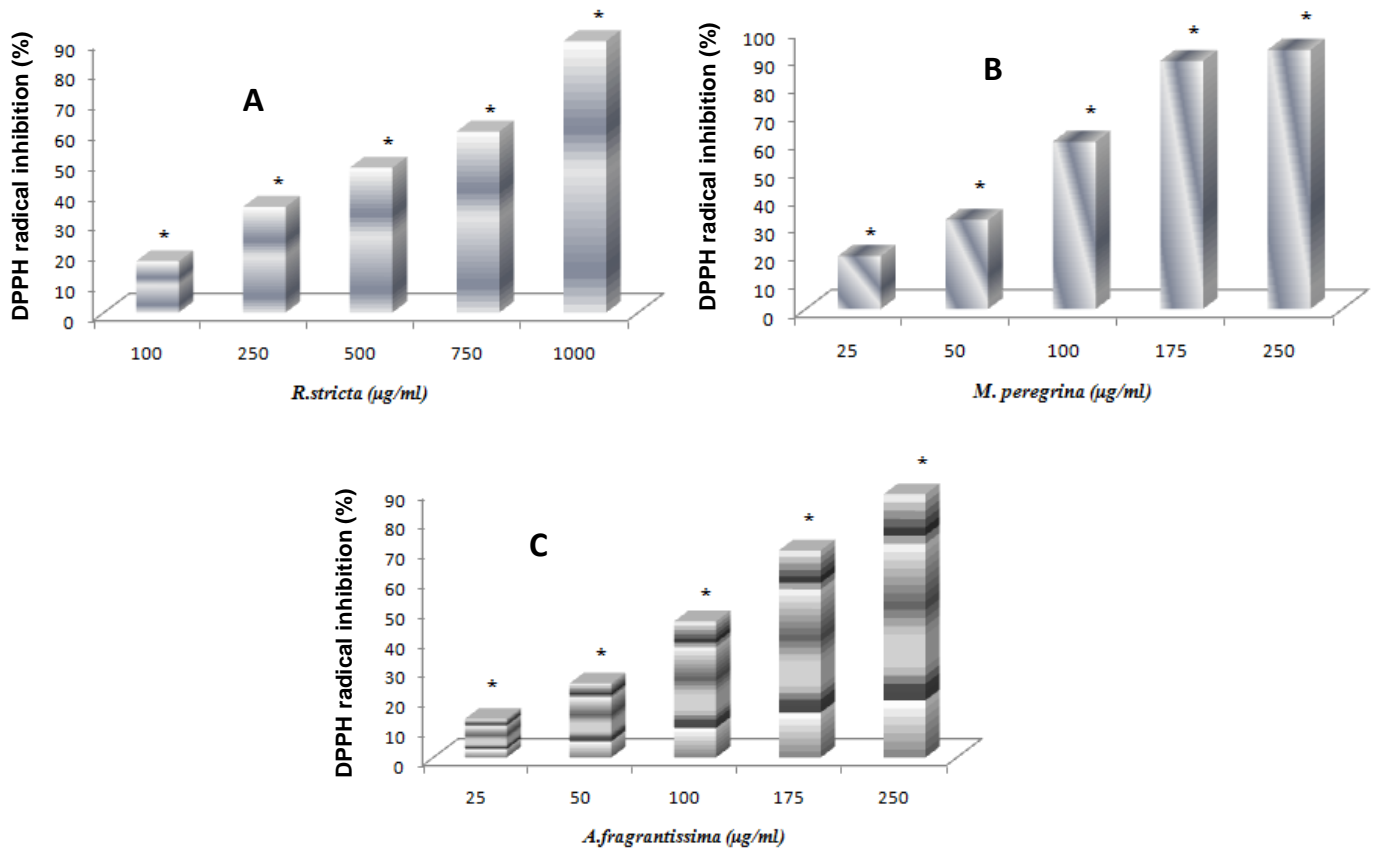
### The tannin contents of the extracts correlate with their ACE inhibition activity

Previous studies have reported a positive association of the tannin contents of various plant derivatives and their anti-ACE properties with tannin molecules acting as non-specific inhibitors of the enzyme (Liu et al., 2003). As shown in Figure 3, the tannin content of the three plant extracts were compared and the result demonstrated the highest to lowest trend as: *R. stricta* > *M. peregrina* > *A. fragrantissima*. As mentioned above, a similar trend has been obtained in the ability of the extracts to inhibit ACE, thereby indicating a degree of correlation in the tannin content with the ACE inhibition potential. ACE is a zinc-containing peptidyl dipeptide hydrolase. It is known that the active site of ACE consist of three parts: a carboxylate binding functionality such as the guanidinium

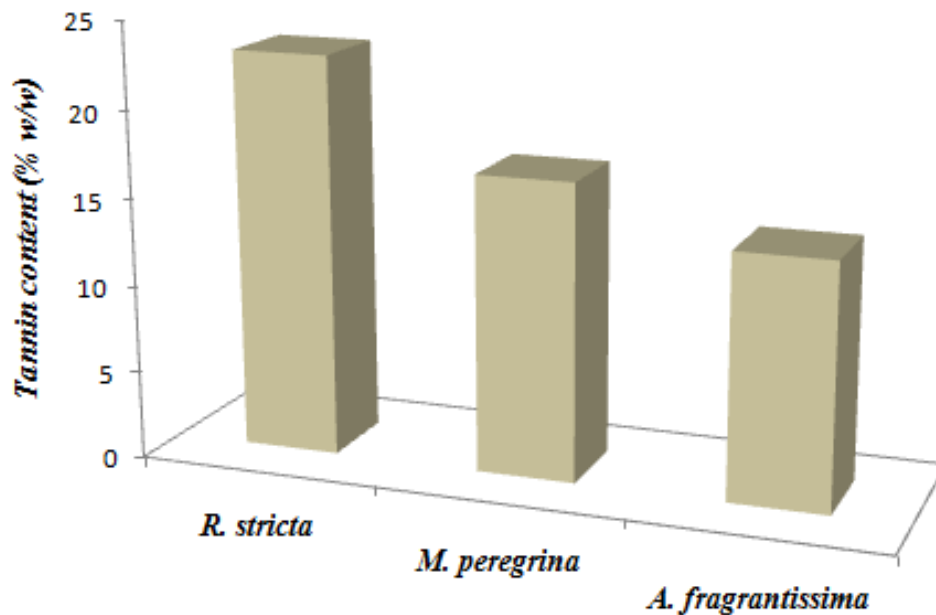
group of Arg, a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues, and a zinc ion. It has been suggested that the anti-ACE activity of polyphenolic compounds might involve the formation of chelate complexes with the zinc atom within the active centre of zinc-dependent metalloproteinases, thereby rendering it ineffective in catalysis (Ojeda et al., 2010).

### Conclusion

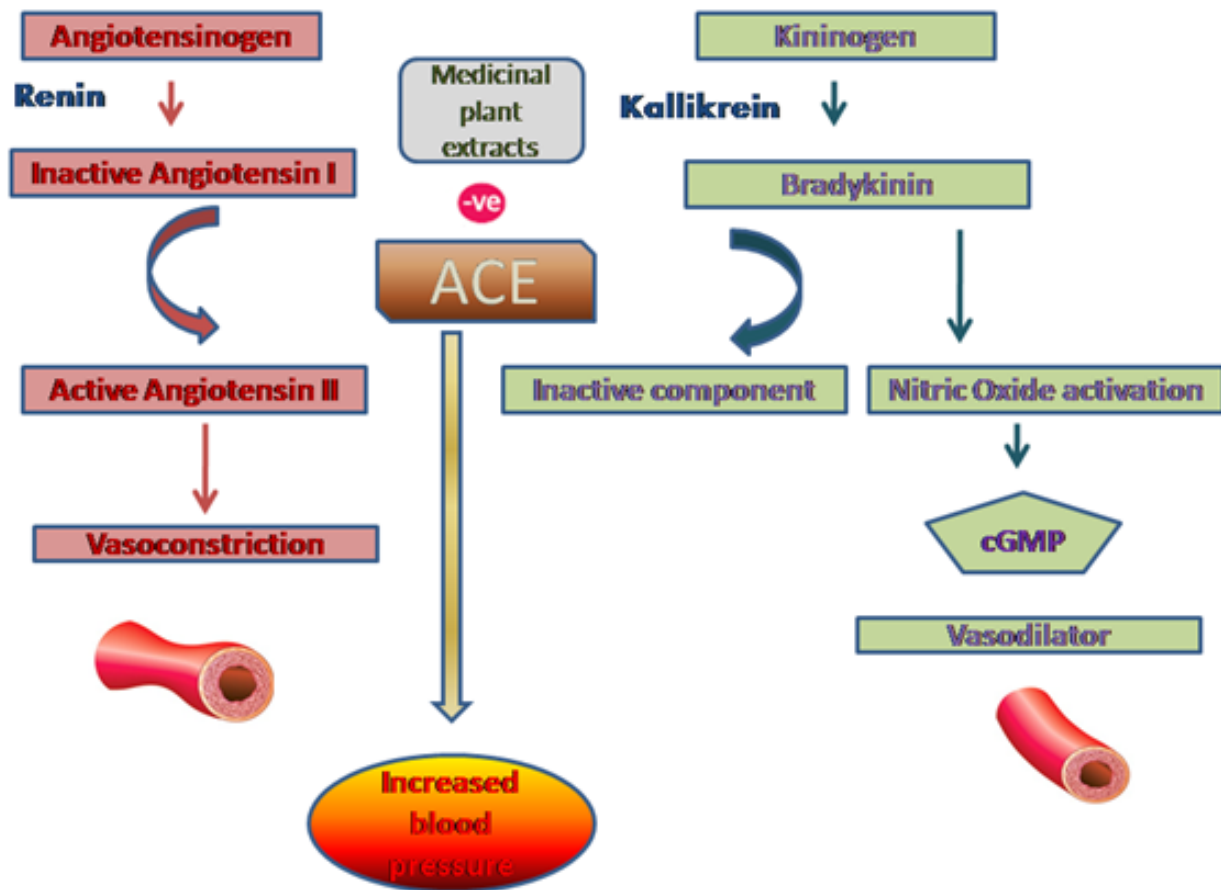
In the early years of the new millennium, about 26.4% of the world's population suffered hypertension, whereas it has been predicted that this statistic would increase by 60% in 2025 (Kearney et al., 2005). Since the proportion of hypertensive population is expected to increase unprecedentedly, new preventive and therapeutic approaches for management of hypertension are essential. The current



**Figure 2.** Radical scavenging capacity of the hydro-methanolic extracts of (A) *R. stricta*, (B) *M. peregrina* and (C) *A. fragrantissima*, expressed by its ability to inhibit DPPH radicals. Values reported are  $\pm$  SEM of three independent experiments. \* $P \leq 0.05$ : significant when compared with the control (in the absence of tested concentrations of extract).



**Figure 3.** Comparative analysis of the tannin contents of the extracts of *R. stricta*, *M. peregrina* and *A. fragrantissima*.



**Figure 4.** Schematic mechanism of the putative potential of the extracts of the tested medicinal plants in the management of hypertension.

study provides three novel sources of plant-derived ACE inhibitors (Figure 4) which could be further investigated for the isolation and characterization of bioactive molecules responsible for the associated pharmacological properties and which might serve as lead molecules in the development of novel anti-hypertensive drugs.

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#### Conflict of interest

The authors confirm that this article content has no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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